

A GENE EXPRESSION AND HISTOLOGIC APPROACH TO STUDY PRODUCTION AND OUTFLOW OF CEREBROSPINAL FLUID IN HINDLIMB SUSPENDED RATS

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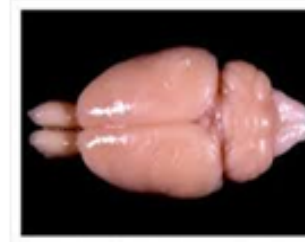
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Objectives and Methods

CSF production in the choroid plexus (CP) and absorption via the arachnoidal villi (AV) may be altered in microgravity and could impact CSF homeostasis in SANS.

Study Aims:

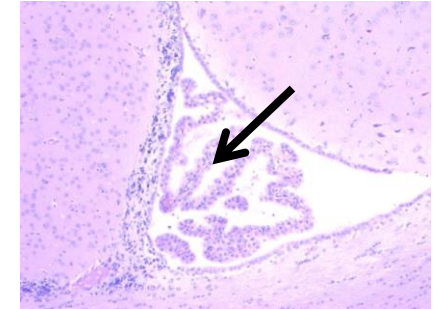
1. Comparative gene expression analysis, ultrastructure and immunohistochemistry of specific proteins in the CP and AV of 9-months old rats subjected to hindlimb suspension (HS) and their normal posture controls.
2. Study of the effects of exposure to 1% CO₂ on the intracranial pressure (ICP) and gene expression of CP in 9-months old rats.



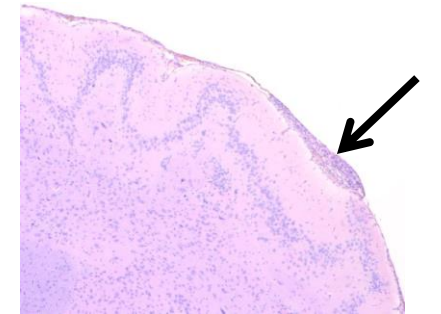
Rat brain



Coronal blocks



Choroid Plexus (CP): H&E stain



Arachnoidal villi (AV): H&E stain

- Frozen tissue
- Laser capture microdissection (LCM)
- RNA isolation
- RNA sequencing

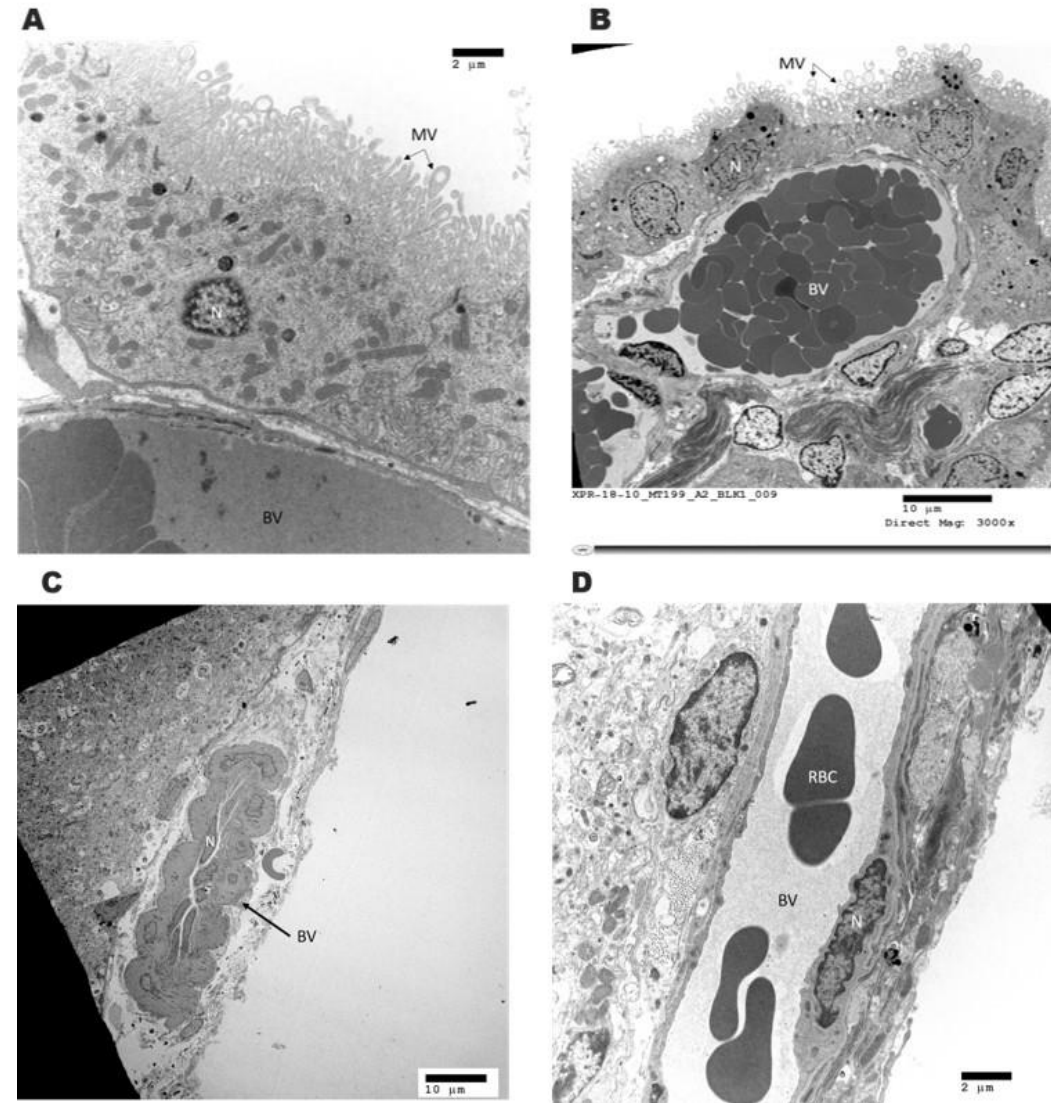
- Bouin fixation
- Immunohistochemistry for AQ4, GFAP and beta-amyloid

- Glutaraldehyde fixation
- Transmission electron microscopy
- Ultrastructure examination

Ultrastructure of Choroid Plexus and Arachnoid Villi by TEM

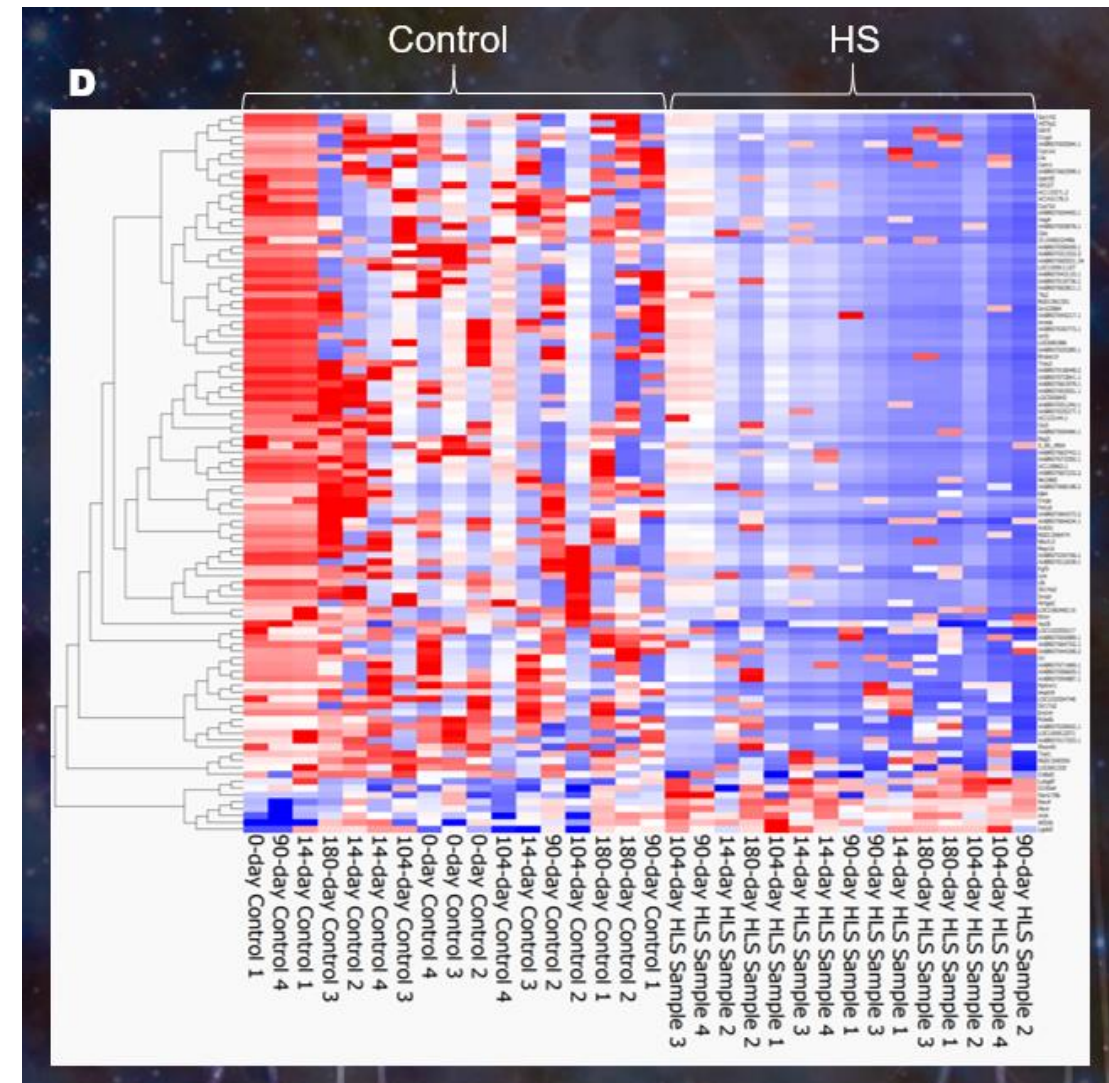
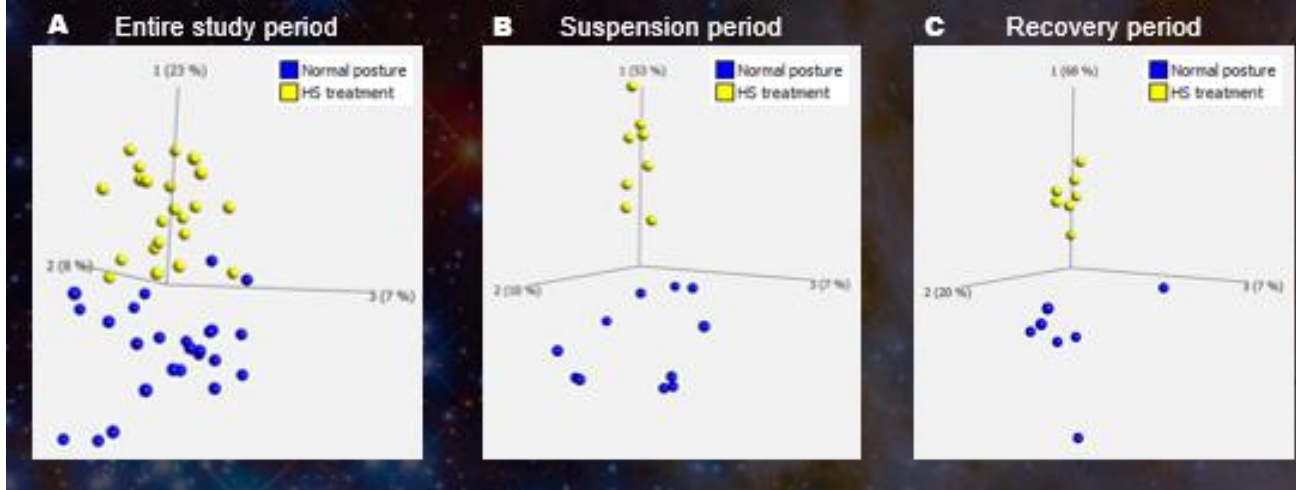
- Sections of glutaraldehyde-fixed brains were obtained by sampling CP from the lateral ventricles and AV from the cerebral convexities.
- Multiple images were obtained using a transmission electron microscope and the structures of interest examined
- Features did not appear to be associated with any of the specific conditions

CP (A, B) showed vascular congestion as well as swelling of the microvilli (B). The arachnoid (C,D) in some of the brains showed vascular congestion and thickening of the vessel walls.

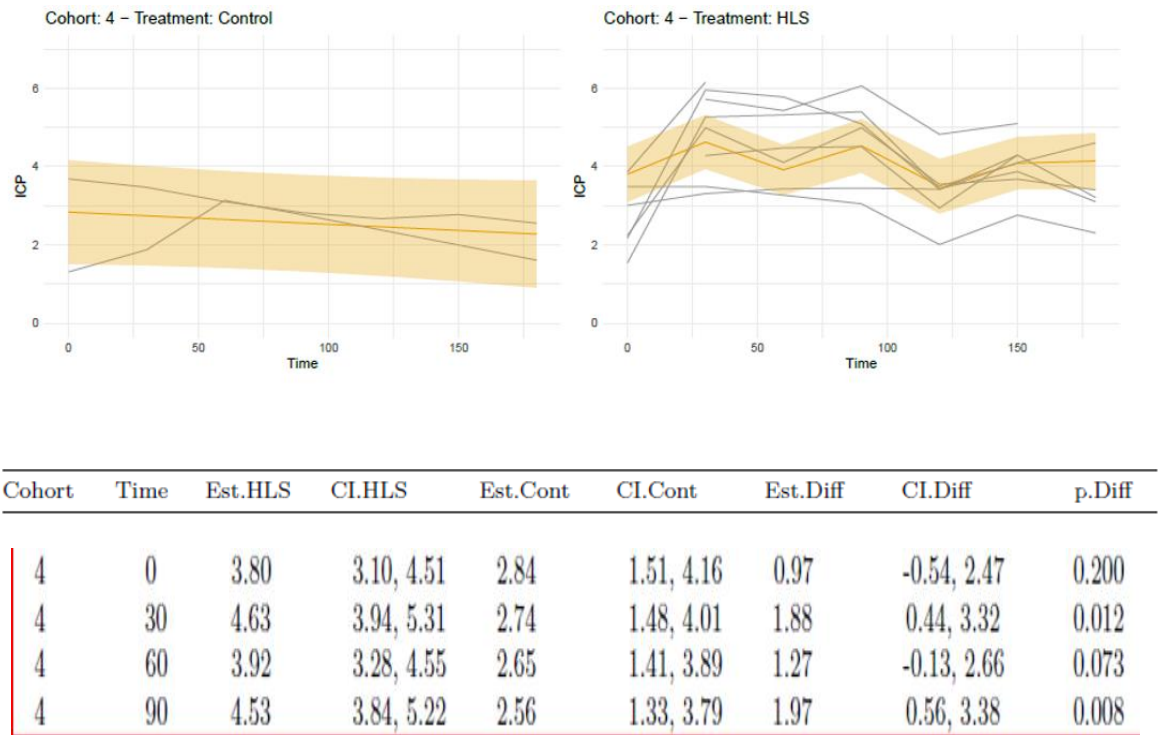


Comparative transcriptomics of the CP in HS and control rats

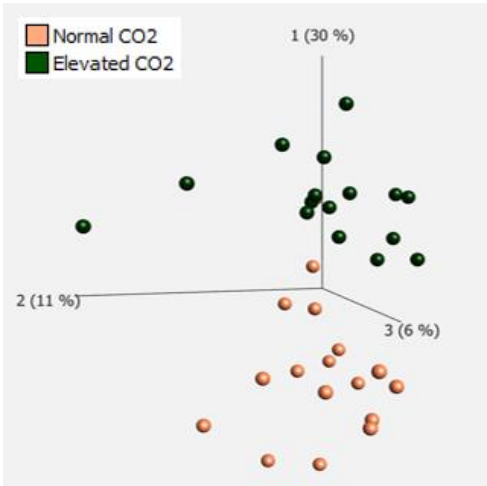
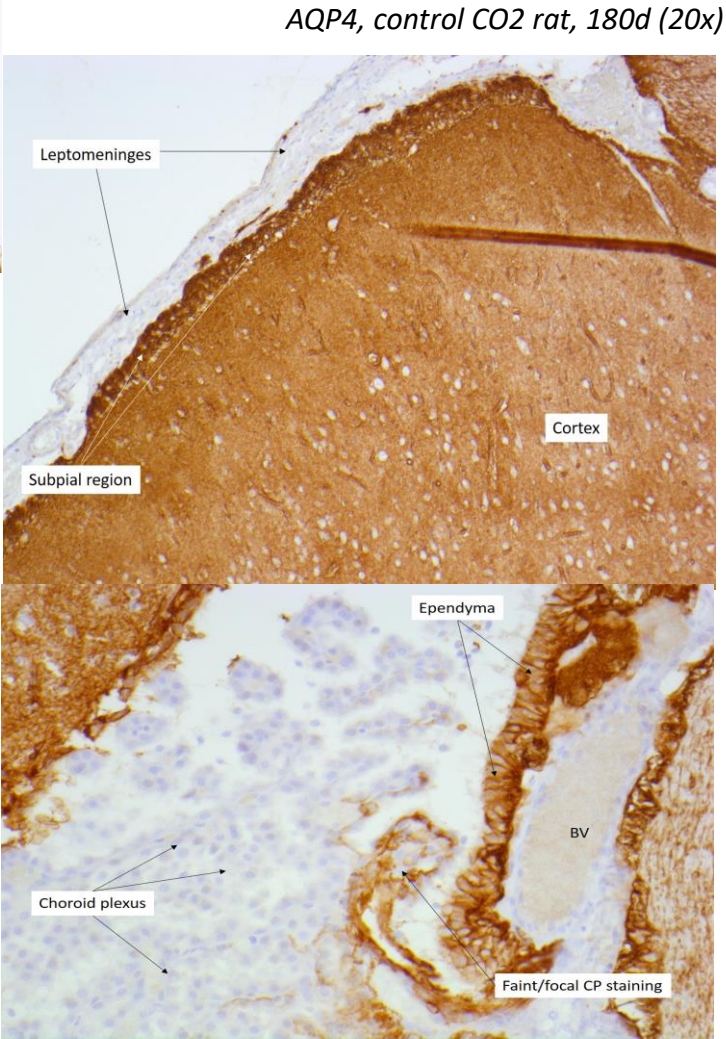
- Gene expression analysis and bioinformatics statistics from RNAseq files done with using Qlucore Omics Explorer v3.3.
- Clear distinction of HS compared to normal posture control samples across all stages of the study, i.e, the suspension and the recovery periods.
- Complete dataset available in Genelab: **GLDS-454: A Gene Expression and Histologic Approach to the Study of Cerebrospinal Fluid Production and Outflow in Hindlimb Suspended Rats**



CO₂ exposure: effects on ICP, gene expression and aquaporin 4 (AQP4)



AQP4, control rat, 90d (4x)



PCA plot showing multigroup comparison of CP gene expression in elevated CO₂ exposed rats versus controls (includes HS and normal posture)